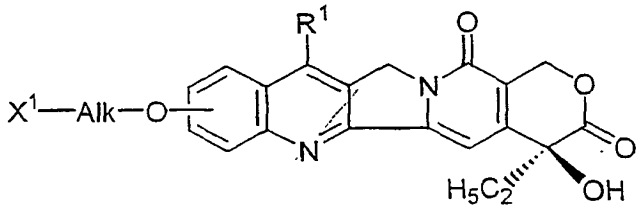
DESCRIPTION

LIQUID PREPARATION COMPRISING CAMPTOTHECIN DERIVATIVE AND PHARMACEUTICAL COMPOSITION PRODUCIBLE BY LYOPHILIZING THE PREPARATION

TECHNICAL FIELD

The present invention relates to a liquid preparation comprising a camptothecin derivative or a pharmaceutically acceptable salt thereof, which shows excellent antitumor activities, a pharmaceutical composition that is producible by lyophilizing said liquid preparation, and a process for preparing said pharmaceutical composition.

More particularly, the present invention relates to a liquid preparation for injection comprising a camptothecin derivative which is prepared by binding a compound of the formula [I]:



[I]

wherein R^1 is a substituted or unsubstituted lower alkyl group, X^1 is a group of the formula: $-NHR^2$ (R^2 is a hydrogen atom or a lower alkyl group) or a hydroxy group and Alk is a straight or branched chain alkylene group

optionally interrupted by an oxygen atom, and a polysaccharide having carboxyl groups via an amino acid or a peptide, or a pharmaceutically acceptable salt thereof, which is adjusted to pH 5-8, or a pharmaceutical composition produced by lyophilizing said liquid preparation, or a process for preparing the same.

BACKGROUND ART

The camptothecin derivatives of the present invention and pharmaceutically acceptable salts thereof are medicinal substances that show excellent antitumor activities against various tumors, especially they show excellent therapeutic effects on solid tumors such as pulmonary cancer, uterine cancer, ovarian cancer, breast cancer, or gastrointestinal cancer (large bowel cancer, gastric cancer, etc.). It has been known that said compounds can be administered parenterally (e.g. intravascular injection) generally in the form of a liquid preparation (e.g. solution, suspension, emulsion, etc.) (JP-10-72467A, EP-0757049A).

DISCLOSURE OF INVENTION

The camptothecin derivative above has the structure wherein a camptothecin compound (active substance) of the formula [I] is bound to a polysaccharide (carboxymethylated dextran or pullulan) through a spacer (an amino acid or a

peptide). Said camptothecin derivatives, when formulated into a liquid preparation, often undergo hydrolysis at the site of spacer or polysaccharide moiety during the preparation process or storage. Hydrolysis of the polysaccharide moiety results in the reduction of the mean molecular weight of said camptothecin derivatives and the increase of the molecular weight distribution, which variation of molecular weight is apt to affect adversely to the pharmacokinetics of said medicinal substance. Further, hydrolysis of the spacer would result in the release of a considerable amount of an active substance (camptothecin compound [I]) at the time of preparation, which is unfavorable in terms of therapeutic effects or side effects. Accordingly, it was desired to find a liquid preparation that is excellent as to drug stability during the preparation process and storage.

The present inventors have intensively studied to solve the problems above, and have found that a liquid preparation with excellent stability can be obtained by adjusting the pH of a liquid preparation comprising a camptothecin derivative of the present invention between 5 and 8 during the preparation process thereof, and have accomplished the present invention.

That is, the present invention provides a liquid preparation for injection comprising a camptothecin

derivative wherein a camptothecin compound of the formula [I] above is bound to a polysaccharide having carboxyl groups via an amino acid or a peptide, or a pharmaceutically acceptable salt thereof, which preparation is adjusted to pH 5-8.

Further, the present inventors have found that a pharmaceutical composition prepared by lyophilizing the liquid preparation above also shows excellent drug stability during the preparation process and storage. Accordingly, the present invention also provides such a pharmaceutical composition.

MODE FOR CARRYING OUT THE INVENTION

In the present invention, any one(s) of camptothecin derivatives disclosed in JP-10-72467A, that is, camptothecin derivatives wherein a camptothecin compound of the formula [I] above is bound to a polysaccharide having carboxyl groups via an amino acid or a peptide can be used. Specific examples of the camptothecin derivatives include those wherein X^1 of a compound [I] and a carboxyl group of an amino acid or a peptide (e.g. a peptide consisting of 2-5 amino acids) are bound to form an acid-amide bond or an ester bond, and an amino group of said amino acid or peptide and a part or all carboxyl groups of a polysaccharide such as a carboxymethylated dextran or

pullulan are bound to form an acid-amide bond(s).

More specifically camptothecin derivatives include those in which a part or all carboxyl groups of a polysaccharide are bound to a N-terminal amino group of an amino acid or a peptide to form an acid-amide bond, and a C-terminal carboxyl group of said amino acid or peptide is bound with X^1 of a compound of [I] to form an acid-amide bond or an ester bond.

Substituents on a compound of a generic formula [I] include the following substituents. When X^2 is $-NHR^2$, a lower alkyl group in R^2 includes a C_{1-4} alkyl group, and a substituent on a lower alkyl group in R^1 includes a hydroxy group optionally protected, a mercapt group and an amino group (e.g. optionally protected by an alkyl group or an acyl group). Alk includes a straight or branched chain C_{1-6} alkylene group which is optionally interrupted by an oxygen atom.

Polysaccharides related to the present invention include a polysaccharide having originally a carboxyl group in its molecule (e.g. hyaluronic acid, pectin, etc.), a polysaccharide (e.g. carboxymethylated pullulan, carboxymethylated dextran, etc.) which is prepared by introducing a carboxyl group into a polysaccharide having originally no carboxyl group in its molecule (e.g. pullulan, dextran, etc.). Among them carboxymethylated dextran (e.g.

degree of carboxymethylation is more than 0.3 and less than 0.8) is especially preferable. Its mean molecular weight is preferably 20,000 - 400,000, especially preferably 50,000 - 150,000.

Preferable camptothecin derivatives are those wherein R^1 is an unsubstituted C_{1-6} alkyl group, X^1 is an amino group and Alk is a straight chain C_{1-6} alkylene group not interrupted by an oxygen atom, a polysaccharide is a carboxymethylated dextran or pullulan, and a peptide is a peptide consisting of 2 - 5 amino acids.

More preferable camptothecin derivatives are those wherein R^1 is ethyl group, a group of the formula: X^1-Alk-O- is 3-aminopropyloxy group, and camptothecin compound [I] bound at position 10 of a camptothecin nucleus and dextran in which a carboxyl group is introduced, are bound via a peptide selected from a group consisting of glycyl-glycyl-L- or D-phenylalanyl-glycine, glycyl-glycine, glycyl-glycyl-glycine, glycyl-glycyl-glycyl-glycine, glycyl-glycyl-glycyl-glycyl-glycine, L- or D-phenylalanyl-glycine, and L- or D-leucyl-glycine. Among those peptides, glycyl-glycyl-glycine is especially preferable.

As pharmaceutically acceptable salts of camptothecin derivatives, alkali metal salts such as sodium salt or potassium salt, alkaline earth metal salts such as calcium salt, or amino acid salts such as arginine salt or lysine

salt are illustrated.

The liquid preparation of the present invention is prepared, for example as follows; (1) a camptothecin derivative above or its pharmaceutically acceptable salt and if necessary other ingredients (e.g. excipients for the pharmaceutical preparations such as buffer, a stabilizing agents) are dissolved in a liquid medium such as water for injection etc., (2) the solution is adjusted to pH 5-8, preferably 5-7.5, more preferably 5-7, especially preferably 6-7 with a suitable buffer (e.g. citric acid, hydrochloric acid, sodium hydroxide, etc.), and then, (3) after diluted with water for injection to get desired drug concentration, the solution is filtered through a membrane filter etc., to remove the insoluble materials (pyrogen etc.) and then is filled into a sealing grass vessel, followed by sterilization to prepare the liquid preparation.

The amount of a camptothecin derivative or a pharmaceutically acceptable salt thereof is not limited, but is 1% (w/v) to 20% (w/v), preferably 1% (w/v) to 10% (w/v).

Buffer used for the liquid preparation of the present invention is selected from the group consisting of citric acid, an alkali metal citrate (e.g. sodium citrate etc.), acetic acid, an alkali metal acetate (e.g. sodium acetate etc.), and an alkali metal dihydrogen phosphate (sodium

dihydrogen phosphate etc.). These compounds are suitably combined to use as the buffer. The preferable combination as the buffer is a combination of citric acid and sodium citrate, a combination of citric acid and sodium dihydrogen phosphate, and a combination of acetic acid and sodium acetate, preferably a combination of citric acid and sodium citrate. Ionic strength of the buffer used for the liquid preparation of the present invention can be adjusted to, for example, 0.01-0.6, preferably 0.01-0.3, especially preferably 0.05-0.2.

To the liquid preparation of the present invention and the lyophilized composition thereof can be added conventional ingredients used for injection as well as the above mentioned ingredients. These ingredients are fillers (lactose, sucrose, mannitol, dextran, maltose, trehalose, etc.), solubilizing agents (polyoxyethylene solbitan fatty acid ester such as polysolbate 80 etc., polyoxyethylen hydrogenated castor oil such as HCO-60 etc, polyoxyethylene alkyl ether such as polyoxyethylene lauryl ether, solbitan fatty acid ester such as Span 80 etc.), stabilizer (alkali metal carbonate such as sodium carbonate, alkali hydrogen carbonate such as sodium hydrogen carbonate etc.), antioxidants (cysteine hydrochloride, tocopherol, ascorbic acid, etc.), tonicity agents (glycerin, glucose, etc.), and preservatives (thimerosal, ethanol, propylene glycol,

benzyl alcohol, para hydoxybenzoic acid alkyl ester such as para hydoxybenzoic acid butyl ester, etc.).

The amount of the filler is, for example, 10-100% to a camptothecin derivative [I] or a pharmaceutically acceptable salt thereof. The amount of the solubilizer is, for example, 0.1-10% to a camptothecin derivative [I] or a pharmaceutically acceptable salt thereof. The amount of the stabilizer is, for example, 0.1-10% to a camptothecin derivative [I] or a pharmaceutically acceptable salt thereof. The amount of the antioxidant is, for example, 0.1-10% to a camptothecin derivative [I] or a pharmaceutically acceptable salt thereof. The amount of the tonicity agent is for example, 0.01-1% to a camptothecin derivative [I] or a pharmaceutically acceptable salt thereof. The amount of the preservative is, for example, 0.001-0.2% to a camptothecin derivative [I] or a pharmaceutically acceptable salt thereof.

The liquid preparation prepared above is filled into a hard vessel such as a sterile ampoule, a vial, a syringe, etc., and is lyophilized by a conventional method to prepare the pharmaceutical composition of the present invention.

The lyophilized pharmaceutical composition of the present invention is prepared as follows.

The amount of the liquid preparation to be filled into

a vessel is, for example, preferably 5-50%(v/v) per the volume of the vessel, especially preferably 10-25%(v/v).

The external temperature on lyophilization is kept preferably at -50 to 60°C, especially preferably -50 to 40°C, and the pressure for sublimation of the solvent used is preferably 0.01-0.2 Torr, more preferably 0.01-0.1 Torr. The rate of lyophilization is preferably adjusted such that the volume of the solvent (calculated into a solution) is sublimated at the rate of 10μl to 100μl per 1cm^2 of the surface area from which the solvent is sublimated for one hour, especially 30μl to 60μl under controlling ingredients of the liquid to be lyophilized, temperature at lyophilization, pressure at sublimation of the solvent, etc.

In case of lyophilizing the liquid preparation, especially the preparation containing mannitol, dextran, and/or sodium carbonate, etc., the breakage of the vessel is protected by previously adding at least one salt selected from the group consisting of alkali metal chlorides (lithium chloride, sodium chloride, potassium chloride, etc.), alkaline earth metal chlorides (magnesium chloride, calcium chloride, etc.) and alkali metal sulfates (lithium sulfate, potassium sulfate, sodium sulfate, etc.), to said liquid preparation. In this case, preferable salts are sodium chloride, sodium sulfate, etc. The amount of said salt is preferably 0.01-10%, more preferably 0.1-5%

per the drug (weight).

The liquid preparation and the pharmaceutical composition prepared by lyophilizing the liquid preparation are preferably stored in a light resistant sealing vessel.

The liquid preparation of the present invention as prepared above, has an excellent property as to drug-stability (a camptothecin derivative) during the preparation process or storage. Therefore, the liquid preparation can be administered directly to a patient. The dosage of the liquid preparation is varied on age, body weight, or condition, but is usually 0.02-50mg, especially 0.1-10mg/kg in calculation to a camptothecin compound [I] (in case of X^1 being $-NHR^2$, its hydrochloride).

The pharmaceutical composition prepared by lyophilizing the liquid preparation of the present invention, has also an excellent property as to drug-stability during the preparation process or storage, and therefore, it is useful for an injection prepared when necessary.

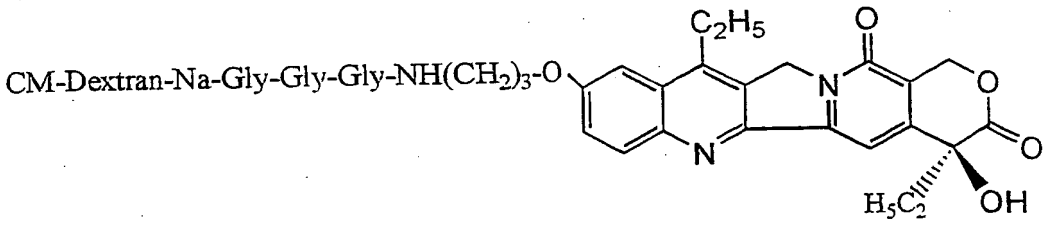
The present invention is further explained in detail by examples, but the present invention should not be limited by these examples.

Example 1

Preparation for liquid preparations

Based on ingredients of Table 1 below, an aqueous drug solution was prepared and filtered through a membrane filter (type: GS, pore diameter: 0.22μm prepared by Millipore Ltd.). The filtrate (1mL) was filled into a grass 3mL-ampoule. Each ampoule was sterilized in vapor at 100°C for 15 min to obtain a liquid preparation.

Drug: Camptothecin derivative described in Example 84 of Jp-10-72467A as represented by the following formula:



wherein CM means "carboxymethylated".

Table 1

	Comparative example	Liq. preparation of present invention			
		1	2	3	4
Drug (g)	0.4				
Sodium dihydrogen phosphate(g)	0.110	0.147	0.180	0.213	0.245
Citric acid	0.118	0.093	0.071	0.047	0.023
0.4M Aq. sodium dihydrogen phosphate solution	q.s.	q.s.			
0.2M Aq. citric acid solution	q.s.	q.s.			
Sodium chloride(g)	0.771	0.771			
Water for injection	q.s.	q.s.			
Total	100mL	100mL			
pH	4.0	5.0	6.0	7.0	8.0

Stability of liquid preparations

The preparation prepared above was preserved under each preservation condition (at 60°C for 20 days, 50°C for 30 days or 40°C for 120 days), and the stability of the drug was tested (Mean molecular weight and molecular weight distribution, and amount of free active camptothecin). The result was shown in the following Table 2. The mean molecular weight of the drug was calculated by GPC multi angles Laser scattering method (MALLS method) and the mean molecular weight distribution was calculated by the following formula:

Mean molecular weight distribution = weight of mean molecular weight (MW)/number of mean molecular weight (MN)

Table 2

	pH	Preservation condition	Mean molecular weight	Mean molecular weight distribution
Liq. preparation 1 of present invention	5.0	Initial	138,900	1.195
		60°C for 20 days	125,800	1.183
Liq. preparation 2 of Present invention	6.0	Initial	129,100	1.169
		60°C for 20 days	131,200	1.177
Liq. preparation 3 of Present invention	7.0	Initial	131,400	1.191
		60°C for 20 days	131,400	1.186
Liq. preparation 4 of Present invention	8.0	Initial	130,900	1.202
		60°C for 20 days	127,000	1.195
Comparative example	4.0	Initial	129,800	1.200
		60°C for 20 days	110,100	1.720

Table 3

	pH	Amount of free active camptothecin compound (%) *			
		Initial	60°C for 20 days	50°C for 30 days	40°C for 120 days
Liq. preparation 1 of present invention	5.0	3.08	13.15	9.17	10.60
Liq. preparation 2 of present invention	6.0	1.67	8.12	5.83	5.53
Liq. preparation 3 of present invention	7.0	1.48	14.53	6.60	6.52
Liq. preparation 4 of present invention	8.0	1.93	17.32	8.31	7.95
Comparative example	4.0	13.81	23.73	24.84	27.33

*: Active camptothecin compound means a compound of the following formula and the amount was quantitatively analyzed by the following conditions (the same hereinafter).

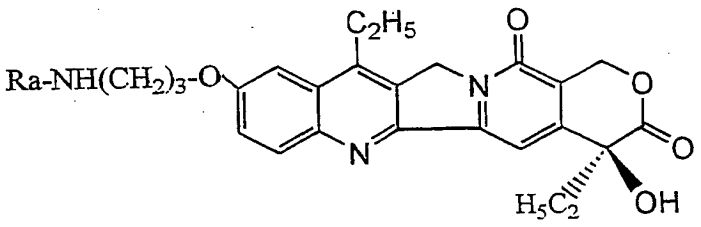
Quantitative analysis: A sample solution was diluted with 0.2M formic acid-ammonium formate buffer in 200 times and then, the diluted solution (0.4mL) and an internal standard solution (0.1mL) were mixed and the mixture was filtered through a membrane filter (pore diameter; 0.45μm) to prepare a test sample for quantitative analysis. The sample was quantitatively analyzed by subjecting to HPLC under the following conditions.

The amount (%) of free active camptothecin in each sample was calculated as 100% of the amount of free active camptothecin compound produced by adding 10 times amount of

6N hydrochloric acid to the sample solution preserved in a refrigerator and then heating at 100°C for 4 hours.

HPLC conditions :

- Column : Inertsil ODS (prepared by GL Science Inc.)
- Mobile phase : 35mM formic acid-ammonium formate buffer (pH3)/acetonitrile=80/20 (flow : 1.0mL/min.)
- Column temperature: 40°C
- Detection : Fluorophotometer (Ex=360, Em=420nm)
- Active camptothecin compound:



wherein Ra is hydrogen atom, Gly-, Gly-Gly- or Gly-Gly-Gly-.

From the result above, in the liquid preparations of the present invention (pH 5-8), decrease of the mean molecular weight of the drug is less in comparison with a liquid preparation of the comparative example and therefore, increase of the molecular weight distribution of the drug was recognized being protected. This reveals that in the liquid preparation of the present invention degradation of the drug(namely cleavage of chain of dextran molecule) can be prevented and undesired formation of free active camptothecin compound due to degradation of spacer portion can be also prevented.

Example 2

Preparation of lyophilized compositions

Using the same drug as the drug of Example 1, and

based on ingredients described in Table 4 each aqueous drug solution was prepared and filtered through a membrane filter (type: GS, pore diameter: 0.22μm prepared by Milipore Ltd.). The filtrate (1mL), was filled into a colorless 13-mL vial and the vial was sealed. Each vial was subjected to lyophilization (pre-freezing: -50°C for 3 hours, primary dehydration: 20°C for 30 hours, secondary dehydration: 60°C for 6 hours) to prepare a lyophilized drug composition.

Table 4

	Comparative example		Composition of present invention			
	A	B	1	2	3	4
Drug (g)	5.0					
Sodium dihydrogen phosphate (g)	0.0059	0.110	0.147	0.180	0.213	0.245
Citric acid	0.153	0.118	0.093	0.071	0.047	0.023
0.4M Aq. Sodium dihydrogen phosphate solution	q.s.		q.s.			
0.2M Citric acid solution	q.s.		q.s.			
Water for injection	q.s.		q.s.			
Total	100mL		100mL			
pH	3.0	4.0	5.0	6.0	7.0	8.0

Stability of lyophilized compositions

The preparations prepared above were preserved at 60°C for 20 days, and the stability of the drug compositions was tested (Change of color, Insoluble materials are present or not after reconstitution, molecular weight distribution of the drug, and amount of free active compound). The result

was shown in the following Tables 5-1 and 5-2.

Table 5-1

[Presence of insoluble materials or not]

	Comparative example		Composition of present invention			
	A	B	1	2	3	4
Change of color	No (yellow)	No (pale yellowish green)	No (pale yellowish green)			
State after reconstitution	Insoluble material: yes	Insoluble material: slight	Insoluble material:no			
pH after reconstitution	3.0	4.1	5.1	6.1	7.1	8.1

Table 5-2

[Change of mean molecular weight and molecular weight distribution of drug]

	pH	Condition of preservation	Mean molecular weight	Mean molecular weight distribution
Composition of present invention 1	5.0	Initial	135,400	1.144
		60°C for 20 days	128,700	1.145
Composition of present invention 2	6.0	Initial	132,800	1.145
		60°C for 20 days	128,500	1.140
Composition of present invention 3	7.0	Initial	130,600	1.143
		60°C for 20 days	128,300	1.147
Composition of present invention 4	8.0	Initial	129,800	1.144
		60°C for 20 days	131,100	1.128
Comparative example A	3.0	Initial	132,900	1.120
		60°C for 20 days	150,300	1.280
Comparative example B	4.0	Initial	135,100	1.134
		60°C for 20 days	138,100	1.209

Table 5-3

[Amount of free active compound]

	pH	Amount of free active compound (%) (Preserved conditions: 60°C for 20 days)
Composition 1 of present invention	5.0	<0.3
Composition 2 of present invention	6.0	<0.3
Composition 3 of present invention	7.0	<0.3
Composition 4 of present invention	8.0	<0.3
Comparative example A	3.0	0.76
Comparative example B	4.0	0.48

Example 3

Preparation of lyophilized compositions

The same drug as example 1 (10g), citric acid monohydrate (0.42g), and sodium chloride (500mg) were dissolved in water for injection (100mL) and the solution was adjusted to pH 5.0 with 1M sodium hydroxide to make the total volume 200mL by adding water for injection. The solution was filtered through a membrane filter (type: GS, pore diameter: 0.22μm prepared by Milipore Ltd.) and the filtrate (2ml), was filled into a colorless grass 3-mL ampoule. Each ampoule was lyophilized by a usual method to prepare a lyophilized preparations prepared when necessary (the preparation of the present invention).

As a comparative example, the same drug (10g) as used in example 1, and citric acid monohydrate (0.42g) were

dissolved in water for injection (100mL) and the solution was treated by the same manner as mentioned above to prepare lyophilized preparations prepared when necessary (Sodium chloride was not added.).

The breakage of the grass ampoules was tested on the composition of the present invention and the composition of the comparative example. The result was shown in the following Table 6.

Table 6

	Broken number per 100 ampoules
Lyophilized composition of the present invention	0
Lyophilized composition of Comparative example	40

Example 4

Preparation of lyophilized compositions

The same drug as example 1 (5g), citric acid monohydrate (0.093g), anhydrous sodium dihydrogen phosphate (0.147) and sodium chloride (50mg) are dissolved in water for injection (50mL) and the solution is adjusted to pH 5.0 with 0.4M aqueous sodium dihydrogen phosphate solution or 0.2M aqueous citric acid solution to make the total volume 100mL by adding water for injection. The solution is filtered through a membrane filter (type: GS, pore diameter: 0.22μm prepared by Milipore Ltd.) and the filtrate (20ml) is filled into a grass 100-mL vial. Each vial is lyophilized by a usual method to prepare lyophilized compositions prepared when necessary.

Example 5

Preparation of lyophilized compositions

The same drug as example 1 (5g), citric acid monohydrate(0.093g), sucrose (5g) and sodium chloride (50mg) are dissolved in water for injection (50mL) and the solution is adjusted to pH 6.0 with 1M aqueous sodium hydroxide solution to make the total volume 100mL by adding water for injection. The solution is filtered through a membrane filter (type: GS, pore diameter: 0.22μm prepared by Milipore Ltd.) and the filtrate (20ml) is filled into a grass 100mL-vial. Each vial is lyophilized by a usual method to prepare lyophilized composition prepared when necessary.

EFFECT OF THE INVENTION

The liquid preparation of the present invention and the composition prepared by its lyophilization have an excellent effect that the degradation of the drug (camptothecin) is less in any stage such as its preparation process, distribution and preservation.